REMARKS

The Office Action dated November 29, 2007, which was in response to Applicant's September 19, 2007 communication wherein Applicant requested completion of the June 22, 2007 Office Action (which, *inter alia,* cited Willett, U.S. Patent No. 6,054,510, without discussing it) pursuant to MPEP 710.06, has been fully considered by the Applicant. Applicant thanks the Examiner for this replacement Office Action that supersedes the June 22, 2007 Office Action.

In the November 29, 2007 Office Action, the Examiner rejected (1) Claims 1-3 and 8-9 as anticipated by Jane (U.S. Patent No. 5,115,000); (2) Claims 4-7 under 35 U.S.C. § 103(a) over Jane in view of Dehennau (U.S. Patent No. 5,510,401) and further in view of Kozma (U.S. Patent No. 6,242,503); (3) Claims 1-17 under 35 U.S.C. § 103(a) over Dehennau in view of Kozma and further in view of Chinnaswamy (U.S. Patent No. 5,496,895); and (4) Claim 18 under 35 U.S.C. § 103(a) over Dehennau in view of Papazoglou (U.S. Patent No. 5,216,075). In response, Applicant has amended independent Claims 1, 10 and 17, amended dependent Claims 4, 8, 9, 16 and 18, canceled dependent Claim 2 and added dependent Claim 19 in order to more clearly distinguish Applicant's synthetic polymer and starch blend and method of synthesizing the blend from the cited references.

Amendments to the claims and new claims were made purely for clarification and do not change the scope of the claims. Applicant respectfully submits that these amendments do not add any new matter and are supported by the original specification. In addition, Applicant asserts that the amendments do not change the scope of the claims and therefore no new search is required. Applicant respectfully requests reconsideration and allowance of the claims. For the

reasons stated below, Applicant believes the foregoing amendments place the application in condition for allowance.

1. CLAIMS 1 AND 3 AND 8-9, AS AMENDED, ARE NOT ANTICIPATED BY JANE

Independent claim 1 has been amended, *inter alia*, to require that the compatibilizer comprise "a polymer and a grafting compound, wherein said grafting compound is covalently bound to said polymer". The Examiner concedes that Jane "does not disclose [a compatibilizer in which] a grafting compound is covalently attached to a polymer." Nov. 29, 2007 Office Action at page 4. Thus, claim 1, and its dependent claims 3 and 8-9, are not anticipated by Jane.

Further, Jane does not teach or suggest using "a granular and unplasticized starch having a moisture content of less than about 1%", as required by amended independent claim 1. (Spec. at page 10, line 21 - page 11, line 4 and at page 16, lines 12-13). While Jane does use a granular starch, Jane's granular starch is merely used as a convenience and is not dried, *e.g.*, column 2, lines 6-12 ("any type of starch may be used," "the term starchy material includes ... modified starches"). As set forth in Chapter 4, *Corn Starch: Properties*, of Technology of Corn Wet Milling and Associated Processes (Paul Harwood Blanchard, 1992), commercial corn starch typically has 12% moisture content. (See *id.* at 141-42.) Nowhere does Jane teach or suggest drying the granular starch, so Jane's starch is inherently understood to have a moisture content considerably higher than 1%.

2. CLAIMS 4-7, AS AMENDED, ARE NOT RENDERED OBVIOUS BY JANE AND DEHENNAU AND KOZMA

The Examiner rejected claims 4-7 as obvious in view of Jane, Dehennau, and Kozma. The Examiner conceded that Jane does not disclose a grafting compound covalently attached to a polymer (as now recited in independent claim 1) or that the "grafting compound is maleic anhydride." Nov. 29, 2007 Office Action at page 4.

The Examiner asserts that Dehennau discloses "a biodegradable film produced from a composition comprising starch, polyethylene modified by grafting maleic anhydride and a non-modified polyethylene", but states that, like Jane, Dehennau does not disclose "the formation [of] a covalent bond between the polymer and maleic anhydride grafted to said polymer." The Examiner relies on Kozma to cure this deficiency.

Dehennau teaches that the starch used in the inventive composition is plasticized. (Col. 3, lines 40-44, col. 4, lines 50-54 (describing "various alloy compositions of the invention, on a common base formula containing 75% by weight of starch + plasticizer and 25% by weight of compatibility-promoting agent"), examples 1-14 (using the plasticizer glycerine in all examples)). The claims, as amended, require the starch to be unplasticized. (Spec. at page 9, lines 16-22). In addition, Dehenneau does not address the low moisture content of the granular starch now recited in the claims.

Kozma does nothing to cure the deficiencies of Jane and Dehennau. While Kozma does disclose "the maleated polyethylene, wherein maleic anhydride-grafting is covalently bonding one or more maleic anhydride groups to the original polymer chains," this is all Kozma discloses of any relevance to the present claims. Kozma "relates to foamed articles and non-foam flexible materials formed from ethylene-vinyl acetate copolymers and maleic anhydride." Kozma has nothing to do with the claimed invention as recited in claim 1 reproduced below and its dependent claims 4-7:

A synthetic polymer and starch blend comprising:

1-30 wt.% a granular and unplasticized starch having a moisture content of less than about 1%;

1-24 wt.% a compatibilizer comprising a polymer and a grafting compound, wherein said grafting compound is covalently bound to said polymer; and

the remainder a second polymer.

There is no teaching, suggestion or motivation that would have led one of ordinary skill to take the maleated polyethylene taught by Kozma and put it into starch-based compositions of Jane or the plasticized starch composition of Dehennau.

Moreover, Dehennau and Kozma do nothing to cure the other deficiencies of Jane, specifically the failure to disclose now-claimed moisture content of the starch as less than about 1%. Claims 4-7 should be allowed.

3. CLAIMS 1-17, AS AMENDED, ARE NOT RENDERED OBVIOUS BY DEHENNAU IN VIEW OF KOZMA AND CHINNASWAMY

The deficiencies of Dehennau and Kozma are explained above. In brief, Dehennau calls for a plasticized starch, while the present claims require the starch to be unplasticized, and Dehennau does not disclose that the moisture content of the starch should be less than about 1%. Kozma's mere disclosure of a maleated polyethylene does not address these deficiencies. The Examiner asserts that Chinnaswamy discloses "biodegradable polymer composition comprising a starch and non-biodegradable plastic, wherein the non-biodegradable polymer is treated by adding an oxidizing agent."

Chinnaswamy's disclosure does nothing to cure the deficiencies of Kozma and Dehennau. Like the other cited references, Chinnaswamy does not require the starch to have a moisture content of less than about 1%, required by amended independent claims 1, 10 and 17. Claims 1-17 are patentable thereover.

4. CLAIM 18, AS AMENDED, IS NOT RENDERED OBVIOUS BY DEHENNAU IN VIEW OF PAPAZOGLOU

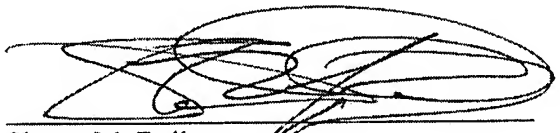
As explained above, Dehennau calls for a plasticized starch, and discloses nowhere that the moisture content of the starch should be less than about 1%. Papazoglou's disclosure of a maleated block copolymer does not cure these deficiencies. Claim 18 is patentable thereover.

In view of the foregoing, Applicant respectfully submits that the independent claims patentably define the claimed invention over the citations of record and other prior art. Further, the dependent claims should also be allowable for the same reasons as their respective base claims and further due to the additional features that they recite.

It is submitted that the application is now in condition for allowance and such action is earnestly solicited.

The Commissioner is authorized to charge any additional fees associated with this application or credit any overpayment to Deposit Account No. 08-1500.

Respectfully submitted,



Shawn M. Dellegar
Registration No. 58,994
Head, Johnson & Kachigian, P.C.
228 West 17th Place
Tulsa, Oklahoma 74119
(918) 587-2000
Attorneys for Applicant

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Technology of Corn Wet Milling

and Associated Processes

by

Paul Harwood Blanchard

Technical Specialist, American Maize-Products Company, Hammond, Indiana, U.S.A.

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Chapter 4

CORN STARCH: PROPERTIES

4.1 BIOSYNTHESIS

4.1.1 Occurrence

Starch, in the form of minute granules, is the major constituent of corn and is found in many other plants. Its exact composition, and the size and shape of the granules, depends upon the plant source (Table 4.1).

The presence of starch in large quantities in plant seeds, such as the corn kernel, provides a food reserve to permit growth to become established in the first few days after germination, at which time the newly developing roots are not yet effective. Such crops form high energy constituents of human foods and animal feed, and, when the starch can be separated easily in a relatively pure form, they provide a source of derived products for food and industrial use.

4.1.2 Granule formation

Corn starch is formed in small bodies, the plastids or amyloplasts, which are part of the cells of the kernel endosperm. Enzyme systems in the amyloplasts are responsible for assembling basic materials into the complicated macromolecules of which the starch granule is composed. At an early stage of development there may be more than one embryo starch granule in each amyloplast, but as they grow, they subdivide in such a way that each daughter amyloplast contains only one granule.

Starch is a polyglucan, which is a polymer formed almost entirely of chains of anhydro-glucose units. The chains are of two basic types, amylose being linear and amylopectin branched, and it seems likely that the synthesis of each component is directed by a separate enzyme system.

The amylose and amylopectin macromolecules are arranged more or less radially in the granule in a semi-crystalline form established by hydrogen bonding (Section 4.3.1), the main structural element being amylopectin. Growth of the granule is effected by

TABLE 4.1

PROPERTIES OF STARCHES

TYPE OF STARCH	CORN (MAIZE)	WAXY MAIZE	AMYLOMAIZE HIGH AMYLOSE
Granule size in microns	Average 15 Smallest 5 Largest 25	Average 15 Smallest 5 Largest 25	Average 10 Smallest 2 Largest 24
Granule shape	Round, polygonal	Round polygonal (as corn)	Round elongated, multiple
Pattern under polarized light	Black cross	Black cross	Black cross, but frequently absent
Approx. amylose/ amylopectin	26/74	1/99	Up to 80/20
Gelatinization temp. range ^{o}C ^{o}F	62/72 144/162	63/72 145/162	67/100+ 153/212+
Total lipid content approx %	0.5	0.3	0.4
Paste clarity	Opaque	Translucent	Opaque*
Paste texture	Short, heavy body	Long, stringy fluid body	Hard gel*
Paste strength under mechanical shear & prolonged heat	Medium	Low	Medium*
Paste viscosity	Medium, pronounced set-back	Medium/high, no irreversible set-back	Medium/low, very pronounced set-back*
Taste & Odor	Low	Low	Low

*High amylose starch will not disperse properly unless pressure-cooked to 150^{o}C (302^{o}F).

TABLE 4.1

PROPERTIES OF STARCHES

TYPE OF STARCH	WHEAT	POTATO	SWEET POTATO
Granule size in microns	Two fractions 2-10 20-35	Variable 15-100	Average 15 Smallest 10 var. Largest 25
Granule shape	Round, elliptical	Egg-like with striations like oyster shell	Polygonal
Pattern under polarized light	Black cross	Irregular black cross	Black cross
Approx. amylose/ amylopectin content	25/75	24/76	18/82
Gelatinization temp. range ^{o}C ^{o}F	52/64 126/147	56/69 133/156	58/74 136/165
Total lipid content approx. %	1.0	Very low	Very low
Paste clarity	Opaque	Translucent	Translucent
Paste texture	Short, heavy	Long, stringy fluid body	Long, stringy fluid body
Paste strength under mechanical shear & prolonged heat	Medium	Low	Low
Paste viscosity	Med/low, pronounced set-back	Very high moderate set-back	High, moderate set-back
Taste and odor	Low	Slight cucumber-like	Low

TABLE 4.1

PROPERTIES OF STARCHES

TYPE OF STARCH	RICE	GRAIN SORGHUM	WAXY SORGHUM
Granule size in microns	Variable 3-8	Average 15 Smallest 5 Largest 25	Average 15 Smallest 6 Largest 30
Granule shape	Polygonal, occurring in clusters	Round, polygonal (as corn)	Round polygonal (as corn)
Pattern under polarized light	Indistinct because of small size	Black cross	Black cross
Approx. amylose/ amylopectin content	17/83	26/74	1/99
Gelatinization temp. range ^{o}C ^{o}F	61/78 142/172	68/75 154/167	67/74 153/165
Total lipid content approx. %	0.4	0.4	0.3
Paste clarify	Opaque	Opaque	Translucent
Paste texture	Short, heavy body	Short, heavy body	Long, string fluid body
Paste strength under mechanical shear & prolonged heat	Medium	Medium	Low
Paste viscosity	Medium/low, pronounced set-back	Medium pronounced set-back	Medium/high, no irrever-sible set-ba
Taste and Odor	Low	Low	Low

TABLE 4.1

PROPERTIES OF STARCHES

TYPE OF STARCH	SAGO	CASSAVA (TAPIOCA)
Granule size in microns	Variable 20-60	Average 20 Smallest 5 Largest 35
Granule shape	Egg-like with some truncated forms	Round, oval indentations
Patter under polarized light	Irregular Black cross	Black cross
Approx. amylose/ amylopectin	27/73	17/83
Gelatinization temp. range °C °F	60/72 140/162	52/64 126/147
Total lipid content approx. %	Very low	0.1
Paste clarity	Translucent	Translucent
Paste texture	Long, stringy fluid body	Long, stringy fluid body
Paste strength under mechanical shear & prolonged heat	Med/low	Low
Paste viscosity	Medium/high, moderate set-back	High, low set back
Taste & Odor	Low	Fruity

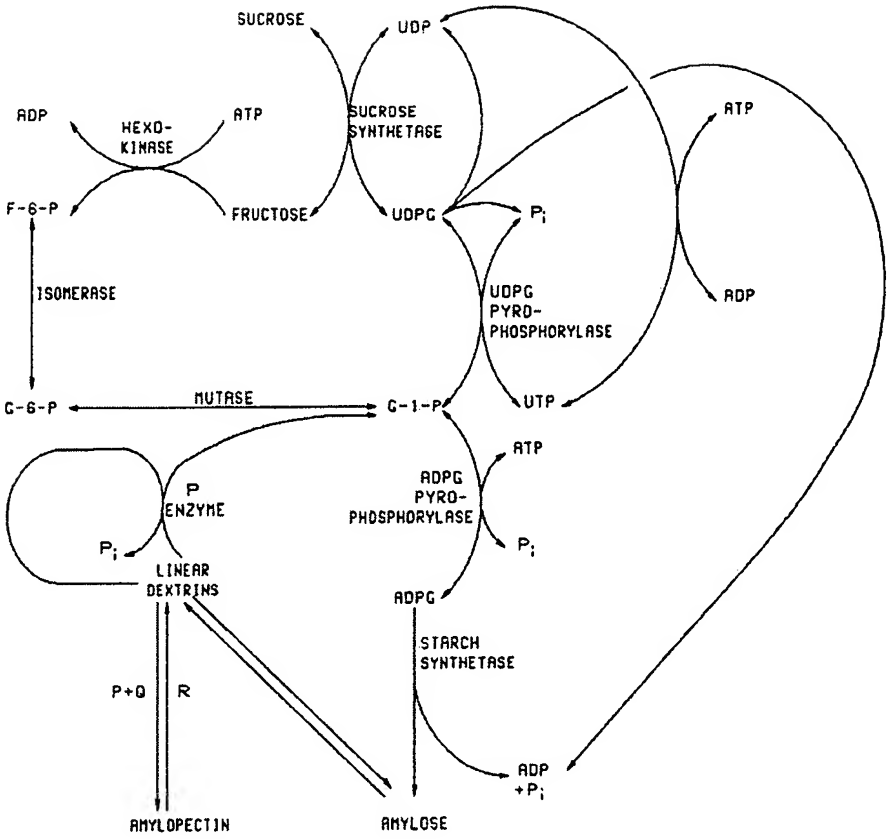


FIGURE 4.1

PROPOSED PATHWAY FOR STARCH SYNTHESIS

FOR KEY SEE TABLE 4.2

TABLE 4.2

ENZYMES PROBABLY INVOLVED IN STARCH SYNTHESIS

NAME	REACTION CATALYZED	REMARKS
Sucrose synthetase	Sucrose + UDP $\longrightarrow$ UDPG + Fructose	
Hexokinase	Fructose + ATP $\longrightarrow$ F-6-P + ADP	
Isomerase	F-6-P $\longrightarrow$ G-6-P	
Mutase	G-6-P $\longrightarrow$ G-1-P	
UDPG pyrophosphorylase	UDPG + P_i $\longrightarrow$ G-1-P + UTP	
ADPG pyrophosphorylase	ADPG + P_i $\longrightarrow$ G-1-P + ATP	
Phosphorylase (P enzyme)	G-1-P + G_n $\longrightarrow$ G_{n+1} + P_i	Forms a linear glucan with alpha 1:4 linkage
Q enzyme	Amylose $\longrightarrow$ Amylopectin	
Starch synthetase	ADPG + G_n $\longrightarrow$ G_{n+1} + ADP + P_i UDPG + G_n $\longrightarrow$ G_{n+1} + UDP + P_i	
R enzyme	Amylopectin $\longrightarrow$ Linear dextrins	A debranching enzyme
D enzyme	$2G_3$ $\longrightarrow$ G_5 + G $2G_4$ $\longrightarrow$ G_7 + G etc. up to about G_{50}	Also called a disproportionating enzyme

KEY TO ABBREVIATIONS:

UDP	Uridine diphosphate
UTP	Uridine triphosphate
ADP	Adenosine diphosphate
ATP	Adenosine triphosphate
F-6-P	Fructose-6-phosphate
G-6-P	Glucose-6-phosphate
G-1-P	Glucose-1-phosphate
UDPG	Uridine diphosphate glucose
ADPG	Adenosine diphosphate glucose
G_n	A glucose polymer with n anhydro-glucose units
P_i	Inorganic phosphate

deposition of layer upon layer, starting from the "hilum", or botanical center of the granules, which can usually be discerned microscopically. Some variation in the properties of the starch in a granule, particularly an increase in the content of amylose, has been observed to occur as the plant approaches maturity and the granule reaches full size.

There is no evidence for the existence of a membrane surrounding the granule, although in dried starch the outer layers of a granule can acquire a denser structure than the inner, due to the loss of more water.

4.1.3 Enzyme reactions in synthesis

Details of the enzymic reactions involved in the synthesis of starch are still the subject of research, and only a brief outline of current ideas can be given here.

It is envisaged that there are three parts to the system:

i. Formation of active "primer" molecules
ii. Building the linear molecular chains by using the primers
iii. Introduction of branches on the chains.

It is thought that the common starch-degrading enzymes (the various types of "amylases") play little if any part in synthesis, since the reaction equilibria favor breakdown. Raw material for starch synthesis is provided by photosynthesis in the form of glucose phosphates and other low molecular weight materials, but is conveyed about the plant mostly in the form of sucrose. Any scheme to describe the process of starch synthesis must therefore commence with the breakdown of sucrose. Figure 4.1 shows one such scheme, based on the work of Geddes and Greenwood (1), and Table 4.2 lists the major enzymes involved.

If this scheme is followed through it will be seen that the "pool" of linear dextrins provides the "primer" material mentioned above. Once formed, possibly by degradation of amylose, this is built up to amylose by phosphorylase or synthetase, and to amylopectin by phosphorylase plus Q enzyme. It is implied that these enzymes are adsorbed on to the surface of the growing granule, thus imposing a stereochemical control on the polysaccharide structures being deposited.

A number of variations on this hypothesis exist, and more work is required to confirm and clarify the synthetic pathways.

4.2 THE GRANULE

4.2.1 Isolation in the laboratory

The preparation of native starch from laboratory samples of corn presents certain problems. Any dry milling procedure results in a certain amount of damage to the starch granules, and a simulation of the normal wet milling process can reduce the starch viscosity because of inadvertent acid modification in the steeping process.

Adkins and Greenwood (2) recommend steeping at 104°F (40°C) for 40-50 hours at pH 6.5 in acetate buffer with .01 M mercuric chloride. This is followed by maceration, slurrying in water and successively finer screening to an aperture size of 75 microns. The starch/protein filtrate is then shaken repeatedly with toluene to denature the protein, which may then be separated by light centrifugation.

Where mercuric chloride might be objectionable, Watson (3) indicates that the brief use of steeping fluid which has 0.10% of sulfur dioxide in the form of sodium bisulfite results in no significant degradation of the starch. After steeping for 24 hours at 122°F (50°C) the corn is milled with water in a blender with blunted blades and the slurry passed through successively finer screens to 325 mesh. The starch is finally purified by "tabling" or by repeated centrifuging in the presence of amyl alcohol.

A quantitative recovery of the starch in corn is unlikely to be achieved by these relatively simple procedures. In particular, some of the small granule starch associated with horny endosperm is likely to be lost in the fibrous debris. Watson et al (4) describe a much more elaborate technique for laboratory simulation of the wet milling process, which recovers starch and by-products in approximately the yields and qualities obtained in commercial plant.

4.2.2 Birefringence

When normal starch granules are observed under the micro scope using polarized light (crossed Nicol prisms) they appear light against a dark background, typically showing a dark "polarization cross" (Figure 4.2) with its intersection at the hilum. This property of "birefringence", strictly the possession of two refractive indices resulting from the polarization of

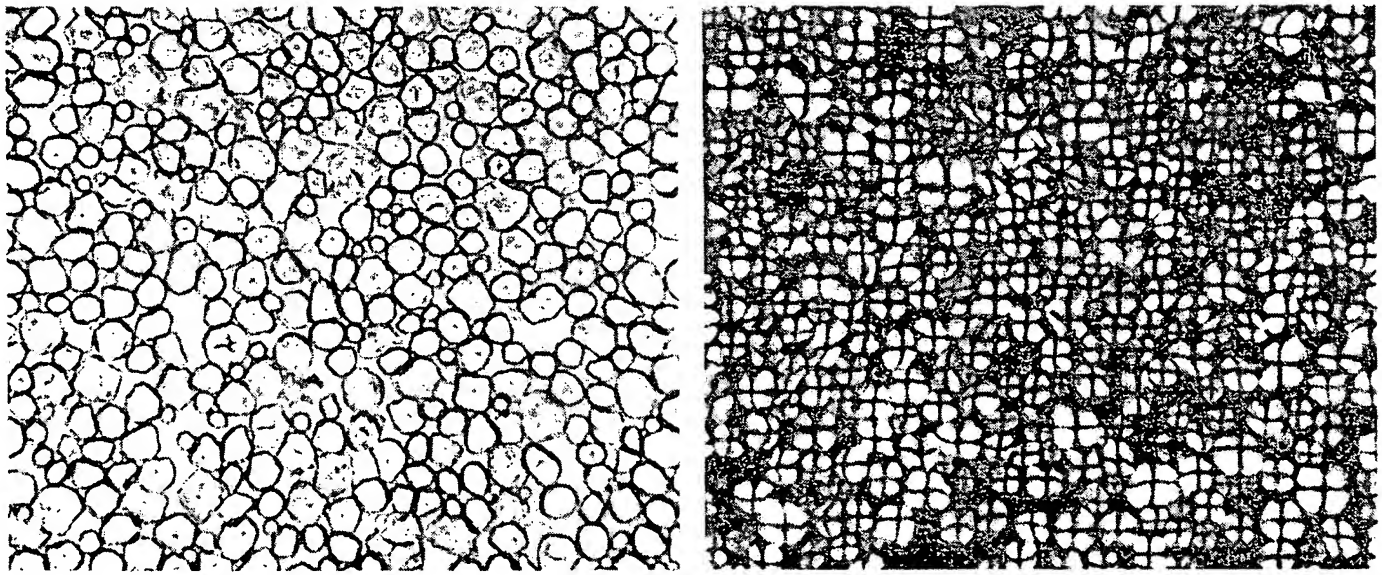


FIGURE 4.2
CORN STARCH GRANULES

(Courtesy of Dr Erik Sorenne, American Maize-Products Co.)

transmitted light, is common in crystalline materials but merely implies that there is some degree of molecular orientation in the granule. The polarization cross is an interference phenomenon due to the spherical shape.

The granules of "waxy" starch, which contain little or no amylose, exhibit normal birefringence, as do granules from which a large part of the amylose has been leached with hot water. The structures responsible must therefore be formed by the amylopectin component. In fact, granules very high in amylose (from amylomaize) often are not birefringent, and have distorted shapes.

The behavior of starch granules in polarized light is sometimes referred to as "anisotropy". This is a more general crystallographic term, but as far as starch granules are concerned it may be assumed to be synonomous with birefringence.

4.2.3 Crystallinity

The molecular detail responsible for crystallinity in a starch granule is not necessarily the same as that conferring birefringence.

Crystallinity is characterized by a fine, regular three-dimensional molecular structure which will produce an x-ray diffraction pattern. Intact starch granules exhibit various types of patterns, depending on the source of starch, moisture level and drying history, and the nature of the responsible structure is not well understood.

In regular corn and waxy maize starch it appears that amylopectin provides the crystalline skeleton, possibly as some type of helical structure. This is a little surprising in view of its branched nature and the difficulty of obtaining crystals in isolated amylopectin.

Amylose, on the other hand, is typically semi-crystalline in isolation. However, only in the case of high amylose starch does amylose appear to play a significant part in the crystallinity of the intact granule.

4.3 CHEMISTRY

4.3.1 Amylose and Amylopectin

As referred to in the earlier sections of this chapter, starch consists of two different types of glucose polymers,

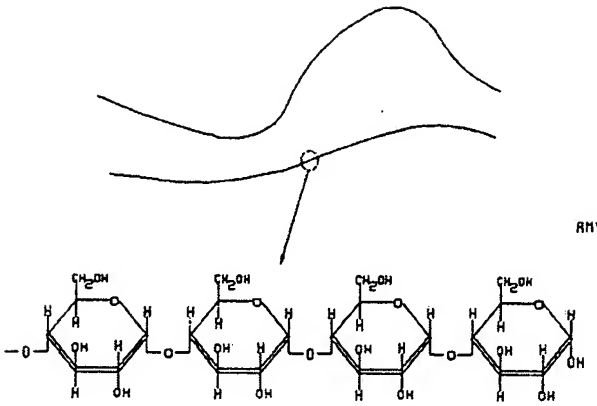
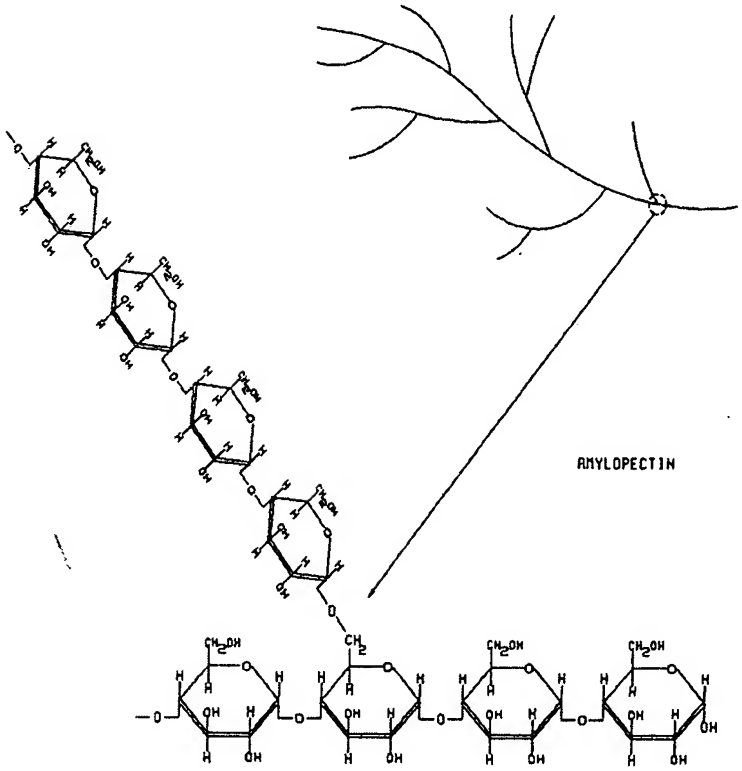


FIGURE 4.3
STARCH COMPONENTS



amylose and amylopectin, with the general formula $(C_6H_{10}O_5)_n$. The predominant linkage between the anhydroglucose units is alpha 1:4, but alpha 1:6 linkages occur in amylopectin, forming branch points as shown in Figure 4.3. The proportions of the two polymers, and hence the physical properties of a particular starch, depend upon its source. Regular corn contains about 26% amylose; waxy corn contains practically none; amylomaize contains up to 80% (Table 4.1).

The term "amylose" describes a mixture of essentially linear polymers, with chain lengths from a few hundred to a few thousand units (average molecular weight up to 600,000 or so). Amylose exists in the form of a relaxed helix which complexes with a number of materials, including iodine and fatty acids (Sections 4.3.3 and 4.3.5). The linear character of the chains facilitates hydrogen bonding, a loose association between hydroxyl groups on adjacent molecules, which, under appropriate conditions, gives rise to the phenomenon of "retrogradation", a form of crystalline precipitation (Section 4.4.4). This property is retained even after partial hydrolytic degradation of the molecule.

In contrast, amylopectin has an extremely large molecule of up to 1,500,000 anhydro-glucose units (molecular weight about 250,000,000). The structure is highly ramified with an average branch length of about 20 units. The branched structure inhibits hydrogen bonding so that amylopectin does not readily precipitate and, in concentrated solution, forms a soft stringy gel on cooling. Amylopectin solutions have an initially high viscosity but are readily degraded by continued agitation.

Where the properties of amylopectin are desired, for example as a base for many modified food starches, it is obtained almost in a pure form by milling waxy maize (Section 1.3.6). Likewise amylomaize (Section 1.3.7) provides starches very high in amylose. These starches are, of course, in granular form.

Some portion of amylose may be leached into solution by treating corn starch granules with warm water; also it may be selectively precipitated as a powder from well dispersed corn starch solutions. However, these are specialized operations, not part of normal wet milling.

4.3.2 Reducing properties

Neither amylose nor amylopectin as such possess any appreci-

able "reducing power". This property, possessed by glucose and fructose and a number of other sugars is exhibited by reaction with alkaline copper solutions, resulting in the precipitation of cuprous oxide.

The active group responsible for reducing power occurs at the 1-carbon position of the glucose molecule, and in a polymer chain all such groups except the end one are blocked by the 1-4 linkages between glucose units. The molecules are so large that the number of available reducing groups at the chain ends is not significant.

However, when the polymer chains are split by hydrolysis more reducing groups become available, and the degree of hydrolysis can be measured by this effect. Expressed as the percentage of glucose (dextrose) that would have the same reducing power, this is the Dextrose Equivalent (D.E.) of a hydrolyzate. Complete hydrolysis to dextrose, never achieved in practice, would result in a 100 D.E. product.

4.3.3 Iodine reactions

Ordinary corn starch in solution reacts with a solution of iodine in potassium iodide to give the characteristic brilliant blue color. Starch granules are also stained by iodine, but often so densely as to appear black.

The coloration is due to an inclusion complex formed between iodine and the amylose molecule, with iodine atoms occupying the center of the polysaccharide helix. The reaction is specific, and the amount of iodine bound under defined conditions may be used as an analytical measure of the amount of amylose present. Amylopectin does not bind any significant amount of iodine under the conditions used, and gives a red coloration, normally masked by the intense blue due to the presence of amylose. Waxy maize starch, which is predominately amylopectin, stains purple.

The helical configuration of amylose also is responsible for complexes with other materials, including lipids which are normally present in corn starch. For quantitative purposes the starch is de-fatted prior to being dissolved for measurement of iodine binding.

The iodine color with amylose varies with the degree of polymerization (DP). When the amylose chain length is reduced by acid or enzyme hydrolysis, the iodine color progresses through

purple, at a DP of 100 or so, to brown, at a DP around 20.

Temperature affects the blue starch iodine color, which disappears as the solution is warmed, possibly due to distortion of the polysaccharide helix, reappearing on cooling.

4.3.4. Optical rotation

Starch solution exhibit strong optical rotation, both amylose and amylopectin having a specific rotation (20°C, sodium D line) of +203°. This provides a convenient method of determining starch in many products, since interfering optically active material is readily washed out of the granular starch prior to gelatinization.

Acid or enzymic breakdown of starch to smaller saccharides can be readily followed by the decrease in optical rotation, the theoretical limit being reached at the specific rotation of dextrose.

4.3.5 Impurities

Commercial starches contain minor amounts of residual impurities, commonly proteinaceous and lipoid compounds.

A typical corn starch has an analysis as follows:

Carbohydrate	99.0% Dry basis
Protein (N x 6.25)	0.35% " "
Soluble Protein	0.010-.015% "
Fat	0.55% " "
Ash	0.10% " "
Phosphorus (as P)	0.01 - 0.02% Dry basis
Moisture	12% Commercial basis

Note that in the wet milling industry "protein" is conventionally measured by multiplying the total Kjeldahl nitrogen content by 6.25. When applied to "soluble protein" this analysis largely represents polypeptides and amino acids.

Some of the protein derives from the matrix in which the starch granules were originally embedded in the corn kernel. The amount of protein present in finished starch may be greater or less depending on the care taken in manufacture, but, with corn starch, it is difficult to reduce this below about 0.25%. Some of this residual may be associated with fatty material and com-

plexed with amylose.

Very little fat can be extracted from starch by normal fat solvents (petroleum ether or carbon tetrachloride) unless the starch is first subjected to acid hydrolysis. However, most of it can be slowly removed from granular starch by extraction with a hydrophilic solvent such as methanol, containing a little water. The process can be speeded up by first ball-milling the starch.

The lipoid material in corn starch is largely present in the amylose fraction and is associated with some phosphorus and nitrogen, although a larger amount of phosphorus is esterified to the starch itself.

Although present in fairly small amounts, such impurities can have a measurable effect on the properties of starches. For example the esterified phosphate in potato starch (0.05 - .10% as P) has a significant influence on the buffering power of hydrolyzates made from it, and de-fatting of corn starch reduces the gelatinization temperature by several degrees and reduces the peak viscosity.

4.3.6 Moisture

Starch in granular form is insoluble in cold water but establishes an equilibrium with moisture in the surrounding atmosphere (5). At room temperature and 50% relative humidity this corresponds to about 12% moisture, which is normally taken as typical. The adsorption phenomenon exhibits hysteresis, i.e. the exact moisture level depends on whether equilibrium is being approached from a moister or a drier state.

4.4 SOLUBILITY

4.4.1 Thermal gelatinization

When a suspension of starch in water is heated, the granules initially swell a little with adsorption of moisture, but retain their birefringence. If heating is continued, a point is reached at which, over a temperature range characteristic of the particular starch, the granules swell abruptly to many times their original size. This "gelatinization" is irreversible and is accompanied by the loss of optical properties, which provides a convenient means of following the change. It corresponds to the point at which the thermal energy is sufficient to overcome the

hydrogen bonding that stabilizes the granular structure.

The granules of a particular starch do not all gelatinize at the same temperature. The range for corn starch is approximately 62° - 72°C (144-162°F), but smaller granules tend to be more resistant to gelatinization and occasionally may require heating to 100°C (212°F) or above. High amylose starches invariably require autoclaving above 100°C for complete gelatinization.

At the point of gelatinization the slurry becomes relatively translucent, and its viscosity increases markedly as the swollen granule aggregates interfere with each other.

As heating continues, particularly if the paste is agitated, some of the swollen granules are disrupted, and hydrated starch passes into colloidal solution, with an accompanying loss of viscosity. A significant degree of dispersion, such as is required for maximum accessibility to enzymic degradation, requires substantial mechanical shear (e.g. homogenizing), or superheating under pressure followed by flashing to atmosphere. Most starch pastes contain swollen granules and granule fragments as well as molecularly dispersed starch.

The gelatinizing temperature range of a particular starch varies somewhat with the conditions of test. The slurry pH can have an effect (Section 4.4.2), but in the range 5-7 it is not significant. The presence of certain chemicals, such as sodium nitrate and urea, lowers the gelatinization temperature, as does esterification and etherification of the starch itself. Other chemicals, typically sodium sulfate, act to increase the gelatinization temperature, presumably by competing for the available water.

Thermal gelatinization is an endothermic process, the "heat of swelling" depending somewhat on the extent of any starch granule damage. Recent measurements (6) indicate a typical value for corn starch of 4.3 cals/gram abs. dry starch (equivalent to 7.7 BTU/lb. dry substance).

4.4.2 Chemical gelatinization

A number of chemicals will disrupt hydrogen bonding and cause swelling and dissolution of starch granules at room temperature. The commonest of these is caustic soda; other reagents with this property include urea, dimethyl sulfoxide and salts such as salicylate, thiocyanate and iodide.

A caustic soda solution of starch is assumed to contain starch broken down to the molecular level (i.e. with no granule fragments), and this is commonly used to establish the "inherent viscosity" of starch. Solutions of starch in dimethyl sulfoxide are often used in the study of starch molecular structure and reactivity.

4.4.3 Acid degradation

In the manufacture of commercial corn syrups, corn starch is gelatinized and simultaneously hydrolyzed by the use of acid, usually hydrochloric acid, at high temperature under pressure. This results in a random hydrolytic splitting of the starch molecules with rapid loss of viscosity and progressive development of reducing sugars. For most purposes, only a partial hydrolysis is required, but in any case it is impracticable to achieve complete degradation because of side reactions which are favored at the high starch concentrations used, and which give rise to isomaltose, gentiobiose and other reversion products. For further details see Section 7.4.

4.4.4 Retrogradation

As described above, corn starch can be obtained in aqueous solution by thermal hydration and suitable dispersion. The solution so obtained is stable if kept above 90°C (194°F), but on cooling undergoes "retrogradation". In dilute solution below 70°C (158°F) this takes the form of an amorphous precipitation of part of the starch; in a solution of 5% or so, a hard gel is produced with further cooling. This phenomenon is largely attributable to the amylose fraction, the linear molecules reassociating by means of hydrogen bonds to give insoluble aggregates. Amylopectin by itself is reasonably stable in dilute solution, and in concentrated solution forms only a soft stringy gel on cooling.

The crystallization of amylose in this way provides a means of separating it from amylopectin. The precipitate is difficult to redissolve, even in boiling water, but dissolves readily in dilute alkali or dimethyl sulfoxide.

This type of retrogradation is inhibited by introducing groups such as acetyl or carboxyl into the starch molecule; presumably the substituent groups interfere with the orientation

of linear amylose which is required to facilitate hydrogen bonding. It is enhanced by a light degree of hydrolytic breakdown, so that acid converted syrups of low D.E. (under 30) are particularly prone to haze formation if allowed to cool during processing, or during storage of the finished syrup. This can be prevented by using bacterial amylase for "destarching", as described in Section 7.2.2.2. Acid-converted syrups are not very uniform in their composition, and the enzyme preferably attacks the larger molecules that tend to reassociate. Most acid-converted syrups above 30 D.E. and most enzyme-converted syrups above about 15 D.E. do not contain molecules of sufficient size to exhibit normal retrogradation.

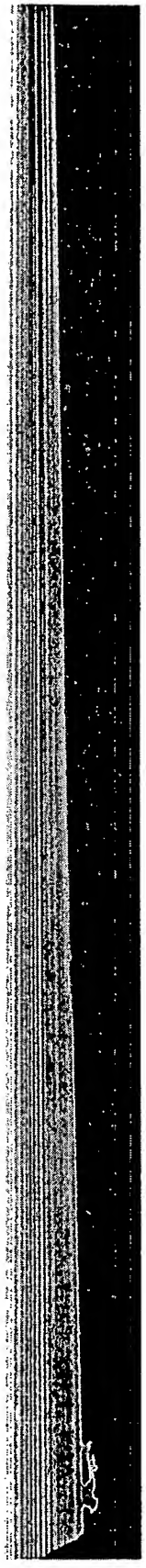
A different phenomenon known as "high temperature retrogradation" (7) occurs when well-dispersed, very low D.E. starch solutions are kept at 75°-90°C (167°-194°F). Under these conditions, pastes prepared by autoclaving untreated starch at 150°C (302°F), or jet cooking starch with temperature-resistant amylase at 95°C (203°F), precipitate an amylose-fatty acid complex in the course of an hour or so. The precipitate takes the form of brittle "granules" 15-40 microns in diameter, which have some degree of crystallinity, exhibiting a polarization cross.

This material might be mistaken for native starch, but the granules are two to three times as large as those of unswollen starch, and have a more rounded appearance; also they are quite susceptible to mechanical damage, to give "pie-slice" fragments.

High temperature retrogradation is inhibited by esterifications (e.g. acetylation) of the starch and by etherification, but introduction of carboxyl groups by oxidation has no effect. Enzyme-liquefied starch loses this tendency to retrograde after a certain degree of hydrolysis, but the exact point is not well defined.

4.5 MODIFICATION

For many applications, both for food and for industrial use, it is advantageous to carry out minor chemical modifications to native starch. Most such modifications are carried out on granular starch in aqueous suspension prior to drying. The dried granular material is superficially similar to regular starch, but the physical characteristics of the cooked product are altered in some respects.



Modification can be carried out to reduce the hot viscosity of the paste, so that higher dry substance levels can be handled, to inhibit retrogradation, to stabilize the paste against mechanical shear or low pH, and so on. Further details are given in Chapter 10.

4.6 EVALUATION

Many routine procedures for chemical and physical analysis of native and modified starches are published by the Corn Refiners Association (8). A few points of particular interest are referred to here.

4.6.1 General properties

For the manufacturer of corn syrups, the important characteristics of starch, which is usually handled as slurry, are the total protein and the soluble protein content. A typical analysis for such a starch is given in Section 4.3.5.

When starch, or modified starch, is used for other purposes, normally as thickener or binder, the viscosity under standard conditions is of critical importance (Section 4.6.3), and total protein, pH, cleanliness, ash, and sulfur dioxide may also be relevant.

4.6.2 Simple Cook

A preliminary evaluation of the characteristics of an unknown starch may be made by a simple cook of a 5% slurry. A standard amount in a beaker is manually stirred with a glass rod while heating in a steam or boiling water bath.

Judgment can be made of the viscosity when hot, the extent of the "string" when the stirring rod is removed from the cook, and the texture and clarity of the paste while hot and on cooling.

Basic paste characteristics for different types of starches are given in Table 4.1, but when possible it is desirable to compare an unknown starch against a known one, cooked simultaneously. Simple modifications will affect the cook; for example an acid-thinned starch is thin when hot but sets up to a hard jelly on cooling, whereas an oxidized starch is thin both hot and cold (see Chapter 10 for further details).

4.6.3 Viscosity

The viscosity of a hot cooked starch paste is due in part to interference between swollen granules and in part to the inherent effect due to large molecules in colloidal solution. This means that the results obtained are very much dependent on the technique of cooking, particularly the amount of shear involved, and on the actual test procedure.

Measurement of "inherent viscosity" avoids the cooking problem by achieving essentially complete (molecular) dispersion with strong alkali (CRA procedure B-61). Even so, the large molecules imparting viscous properties are susceptible to breakdown on further agitation, and the measuring technique, using a U-tube viscometer, must be carefully standardized.

Variations of the "inherent viscosity" test (9) are in common use for control of starch modification processes where the molecular chains are being split by the use of acid or by oxidation. Such measurements are made by timing the flow through a calibrated funnel orifice, and are expressed as an "alkaline fluidity" on a scale where 0 corresponds approximately to unmodified starch and 100 corresponds to water.

The more functional "hot paste viscosity" is measured in many different ways, any one of which may give useful comparative results if the technique is sufficiently well standardized.

The "Scott" procedure has been widely used for regular and lightly modified starches. This technique employs mechanical stirring of a starch slurry in a metal beaker, using an insulated water bath maintained at boiling temperature by live steam. After cooking, the hot paste is placed in the "Scott cup" maintained at bath temperature, and the time for 100 mL to flow through the orifice is measured. The number of seconds taken is the "Scott viscosity"; regular starch falls in the range 70-120.

Much more comprehensive information on the properties of a starch is obtained by using the Brabender Viscoamylograph (10). In this instrument (Figure 4.4) the viscosity of a starch slurry is measured and recorded continuously as it is automatically cooked and subsequently cooled (CRA Method B-9). Agitation is provided by a number of fixed pins in the rotating sample cup, which work in conjunction with pins mounted on a sensor loaded by a torsion spring. A higher viscosity causes more drag, with a consequently greater deflection of the sensor, and this is re-

corded on a strip chart.

During the cooking cycle, the temperature is initially raised at 1.5°C per minute by a programmed thermoregulator which controls a heating jacket. At a suitable maximum, usually 95°C, the temperature is held for a few minutes with continued agitation. The thermoregulator is then put into reverse, and the sample is cooled.

The viscosity trace (Figure 4.5) achieved during a program of this sort provides an evaluation of the sample in several respects:

1. The point on the trace at which the viscosity increases abruptly gives a measure of gelatinization temperature. This basically depends upon the type of starch, but it is lowered by certain hydrophilic modifications, such as oxidation or acetylation, and by the presence of strong alkali or certain other chemicals. It is raised by cross-linking modification; in the presence of certain salts such as sodium sulfate and sodium chloride; and in the presence of a large amount of sucrose.

2. The peak viscosity shown on the trace is a measure of the "strength" of the starch. It can be adversely affected by poor processing conditions, for instance excessively long steeping, or by artificially drying the corn at too high a temperature, so that kernels exceed 140°F.

3. The extent to which the viscosity of the paste resists break-down on continued agitation at temperature indicates the stability that can be expected of the starch when in use. A starch such as waxy maize, which has a very high viscosity peak, tends to break down substantially; when cross-linked by modification the peak is reduced and the stability is improved.

4. The viscosity of a cold paste bears no particular relationship to the hot viscosity, but is indicative of the type of starch and any treatment it may have received in manufacture. For example, a light acid treatment of corn starch reduces its hot viscosity, but may actually increase the cold viscosity owing to a more pronounced tendency to retrograde.

It should be noted that if a starch actually forms a solid gel during test the results are not meaningful as the gel will fragment. It should be tested at a lower concentration.

FIGURE 4.4

BRABENDER VISCOAMYLOGRAPH

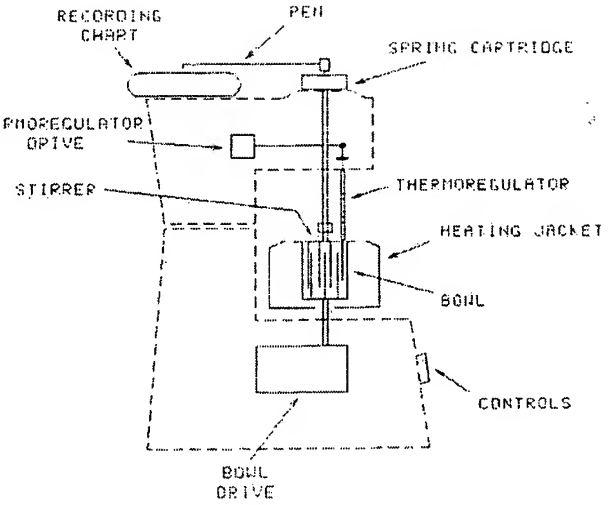
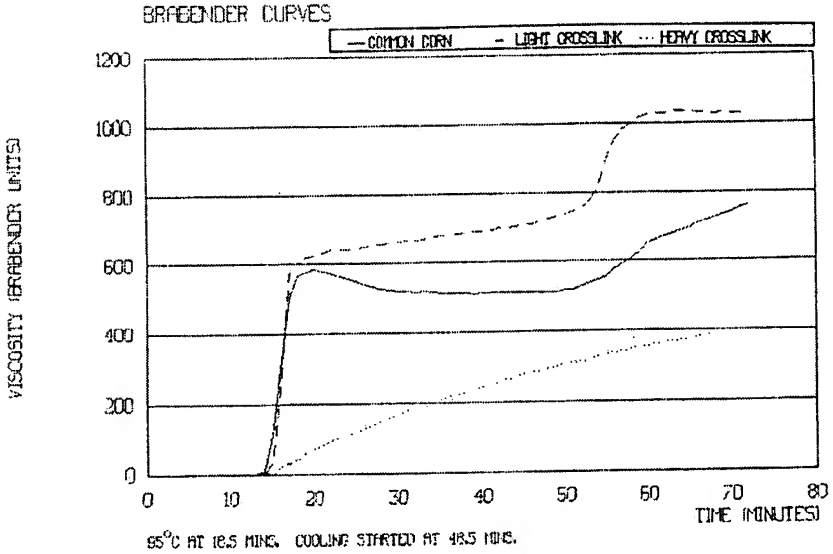


FIGURE 4.5

BRABENDER CURVES



95°C AT 18.5 MINS. COOLING STARTED AT 48.5 MINS.

4.7 MICROSCOPY

Routine microscopic examination of a starch may include its appearance in normal and polarized light, the gelatinization characteristics and the effect of staining with iodine or specific dyes (11).

This requires a microscope with magnifying power up to about 450X, with polarizing attachment (Nicol prisms), a calibrated eyepiece micrometer and a hot stage (Kofler or similar). A hemocytometer counting chamber is also of value for some observations.

4.7.1 Granule size and shape

The type of starch present in an unknown sample can often be determined from the appearance of its granules. Samples are conveniently studied in an aqueous suspension of about 0.2% and comparison made with the descriptions given in Table 4.1 and with genuine samples of known starches.

As shown in Figure 4.2, many corn starch granules are roughly round in shape, but there are also a number which are polygonal, resulting from areas of denser packing in the kernel. Granules are from 5-25 microns in diameter, the weight average being about 15. In many cases the hilum, or botanical point of origin of the granule, is visible as a small spot, and sometimes minute cracks can be seen radiating from this point.

Sometimes the factory drying process causes granules to clump together. Generally, these aggregates disperse in water, and, when this phenomenon is of particular interest, the starch should be slurried in glycerol for examination.

Glycerol may also be used as a mounting medium for pregelatinized starches, which swell and become very viscous in aqueous slurries. However, a satisfactory identification of the typical starch from which such material was made usually depends on finding a few ungelatinized granules. This is facilitated by thinning a warm aqueous sample suspension with liquefying amylase and examining the remaining sediment.

4.7.2 Effect of polarized light

As described in Section 4.2.2, most starch granules are birefringent and exhibit a polarization cross when examined in the dark field produced by partially cross Nicol prisms. This

characteristic is useful in identifying starch granules in mixture with other materials, but is of limited value for identification of the type.

4.7.3. Gelatinization temperature range

It was mentioned in Section 4.4.1 that, when starch is heated in aqueous suspension, the granules swell extensively and lose the polarization cross. Thus, a microscope equipped with a hot stage provides a sensitive means for determining gelatinization temperature.

As described by Watson (12), a prepared slide of a given starch sample is gradually heated (2°C/min) while being observed under partially crossed Nicols. It will be seen that individual granules gelatinize quite sharply, but the temperature varies from granule to granule over a range which is characteristic of the particular starch species. 62°-72°C (144°-162°F) is typical for regular corn.

For accurate comparisons, the percentage of granules gelatinized at each temperature can be plotted, but for most purposes the temperature values at which 2%, 50% and 95% gelatinization takes place are sufficient.

The gelatinization temperature range is affected by modifications of the starch, or by incorporating salts or sugars into the cook. Table 4.3 gives a selection of values quoted by Schoch and Maywald (13). When a particular solute raises the gelatinization temperature, as for high levels of sucrose, it may be assumed to be competing for the available water. When it lowers it, as for sodium hydroxide, it appears that the reagent destroys the hydrogen bonds essential to the granular structure.

4.7.4 Staining

The most common "stain" used in starch work is iodine solution, which may be used to identify starch granules or fragments in admixtures with other materials and to distinguish blue-staining regular starch from the red-staining waxy type.

Iodine is often available as a 1% complex solution in 5% potassium iodide. A drop of such stain is placed in contact with the edge of an aqueous mount of the starch under a cover slip and is drawn into the sample by touching a piece of absorbent paper to the opposite edge of the cover slip. This technique presents

TABLE 4.3

GELATINIZATION TEMPERATURE OF VARIOUS STARCHES °C

Corn	62-72	Rice	61-78
Waxy maize	63-72	Grain sorghum	68-75
High amylose	67-100+	Waxy sorghum	67-74
Wheat	52-64	Sago	60-72
Potato	56-69	Tapioca	52-64
Sweet potato	58-74	Barley	51-60

Acid modified corn starch:	40 fluidity	62-72
	60 fluidity	63-73
	80 fluidity	68-77
Oxidized corn starch:	low conversion	55-73
	medium conversion	54-70
	high conversion	52-68
Cross-linked starches:	Corn	62-74
	Waxy maize	63-76
	Waxy sorghum	67-77
Cationic starch:	.046 degree of substitution	52-65
Hydroxyethyl sorghum starch:	.06 degree subs.	61-68
	.10 " "	58-67
Corn starch in sucrose solution, % :	5	61-72
	10	60-74
	20	65-78
	30	70-81
	40	72-85
	50	76-91
	60	84-97
Corn starch in NaOH solution, %	0.2	56-70
	0.3	49-65
Corn starch in Na_2CO_3 solution, %	5	64-75
	10	67-76
	20	78-87
	30	92-103
Corn starch in NaCl solution, %	1.5	68-77
	3.0	70-79
	6.0	75-83
Corn starch, heat-moisture treated by refluxing in diacetone alcohol		68-76

a useful range of staining densities at the advancing boundary of the stain; it will be observed that heavy staining obscures the polarization cross of whole granules, and that partially swollen granules, which do not present a polarization cross, require a high concentration of stain to show significant color.

Routing testing of starch for "waxy or non-waxy" in a mixture (CRA procedure B-64) involves suspending the starch in a more dilute iodine solution (about .04%) and then counting the blue-staining and red-staining granules in a hemocytometer field.

Dyes may be used to establish the ionic character of unknown starches. For example, Methylene Blue, which is a positively charged (cationic) dye will stain anionic starches, which include oxidized starch, phosphate esters and potato starch (a natural phosphate ester). The washed starch is suspended in a 0.1% solution of the dye for a few minutes, washed free of excess dye and examined.

Likewise, cationic starches, prepared by the incorporation of substituted ammonia groups, are stained by negatively charged anionic dyes (e.g. Light Green SF Yellowish). In all such cases, it is helpful to compare an unknown starch with a known sample, tested in the same way.

REFERENCES

1. W. F. Geddes and C. T. Greenwood, Die Staerke, 21 (1969) 148.
2. G. K. Adkins and C. T. Greenwood, Die Staerke, 18 (1966) 213.
3. S. A. Watson, in R. L. Whistler (Ed.,), Methods in Carbohydrate Chemistry, Academic Press, New York, N.Y., 1964 p. 3.
4. S. A. Watson, E. H. Sanders, R. D. Wakely and C. B. Williams, Cereal Chemistry, 28 (1951) 105.
5. Corn Refiners Association, Critical Data Tables, 3rd Edition, 1975, p. 231.
6. M. Woolton and A. Bamunurachchi, Starch/Staerke, 31 (1979) (6) 201.
7. T. Davies, D. C. Miller and A. A. Procter, Starch/Staerke, 32 (1980) (5) 149.

8. Standard Analytical Methods of the Member Companies of Corn Industries Research Foundation, Corn Refiners Association, Washington, D.C., 6th Edition 1980.
9. W. R. Fetzer and L. C. Kirst, Cereal Chemistry, 36 (1959) 108.
10. W. C. Shuey and K. H. Tipples (Ed.), The Amylograph Handbook, American Association of Cereal Chemists, St. Paul Minnesota, revised 1982.
11. G. E. Moss, in J. A. Radley (Ed.), Examination and Analysis of Starch and Starch Products, Applied Science Publications, Ltd., London, 1976.
12. S. A. Watson, in R. L. Whistler (Ed.), Methods in Carbohydrate Chemistry, Academic Press, New York, Volume IV, 1964.
13. T. J. Schoch and E. C. Maywald, in R. L. Whistler and E. F. Paschall (Ed.), Starch: Chemistry and Technology, Academic Press, New York, Volume II, 1967.

FURTHER READING

N.P. Badenhuizen, in R. L. Whistler and E. F. Paschall (Ed.), Starch: Chemistry and Technology, Academic Press, New York, Volume I, 1965.

W. Banks and C. T. Greenwood, Starch and Its Components, John Wiley & Sons, Inc., New York, 1975.